## A rapid micropreparation technique for extraction of DNAbinding proteins from limiting numbers of mammalian cells

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We have developed a simple and rapid method for preparing DNA-binding protein extracts from mammalian cells. The protocol is derived from the large scale procedure of Dignam et al. (1) and utilizes hypotonic lysis followed by high salt extraction of nuclei. The technique described by Dignam has several drawbacks, including the need for large numbers of cells and lengthy incubation and dialysis steps. It is labor-intensive and precludes preparation of multiple samples simultaneously. Our aim in developing this micropreparation procedure was to easily and rapidly extract DNA-binding proteins from small numbers of cells. Frequently, the quantity of cells available for extraction of DNA-binding proteins is limiting, as in analysis of clinical samples, of multiple clones of transfected cells, or of COS cell pools transiently transfected with a cDNA expression library. Ideally, such a technique would allow processing of many samples simultaneously and quickly on the benchtop. The method described in this report accomplishes these goals. In addition, it gives an excellent yield of DNA-binding proteins, comparable to that of the large scale Dignam protocol with minimal proteolysis.

We typically start with between  $5 \times 10^5$  and  $10^7$  cells. All centrifugations of less than 30 seconds are carried out in a room temperature microfuge; between steps, the samples are placed on ice. Adherent cells are scraped into 1.5 ml of cold phosphatebuffered saline (PBS); non-adherent cells are pelleted and resuspended in 1.5 ml cold PBS. The cell suspension is then transferred to a microfuge tube. Cells are pelleted for 10 seconds and resuspended in 400 µl cold Buffer A (10 mM HEPES-KOH pH 7.9 at 4°C, 1.5 mm MgCl<sub>2</sub>, 10 mM KCl, 0.5 mM dithiothreitol, 0.2 mM PMSF) by flicking the tube. The cells are allowed to swell on ice for 10 minutes, and then vortexed for 10 seconds. Samples are centrifuged for 10 seconds, and the supernatant fraction is discarded. The pellet is resuspended in 20-100 μl (according to starting number of cells) of cold Buffer C (20 mM HEPES-KOH pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM MgCl<sub>2</sub>, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.2 mM PMSF) and incubated on ice for 20 min for high-salt extraction. Cellular debris is removed by centrifugation for 2 minutes at 4°C and the supernatant fraction (containing DNA binding proteins) is stored at -70°C. The yield is 50 to 75  $\mu$ g protein per 106 cells.

Figure 1 illustrates how this micropreparation technique was used to generate DNA-binding protein extracts from approximately 10<sup>6</sup> cells from various murine, simian and human cell lines. The time required from start of the preparation to the loading of the gel shift gel was approximately 90 minutes. Retardation assays were performed as previously described (2). All of the extracts prepared by the microprep technique contain

an ubiquitous protein producing a strong band shift with the NIP probe. The purity of the preparation can be compared between the microprep extract from MEL cells (lane 2) and the extract made by the large-scale Dignam preparation (lane 1). The band shift patterns are identical.

We have used this technology to screen COS cells transiently expressing a cDNA library for specific DNA-binding proteins and to screen stably-transfected cells for expression of DNA-binding proteins. The speed of the assay has also permitted us to use it for preparation of transcription factors from aliquots taken at multiple time-points after treatment of a population of cells with various agents.

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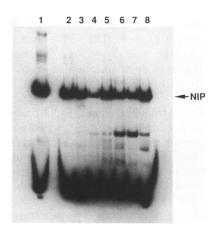


Figure 1. Gel shift assay using DNA-binding protein extracts generated by the micropreparation technique. Band-shift assays were performed using 6  $\mu$ g of each protein sample and a radiolabeled NIP binding site probe from the interleukin-3 promoter (2). Lane 1 shows gel-shift patterns from nuclear extracts prepared by the traditional Dignam protocol. Lanes 2–8 show band-shift patterns from nuclear extracts obtained using the micropreparation technique from murine erythroleukemia cells (MEL), NIH-3T3 cells, NS-1 murine myeloma cells, HTLV-I transformed human T cells (SPP), COS cells, HeLa cells, and human peripheral blood T lymphocytes, respectively.